# THE
# BOTANICAL GAZETTE

## *OCTOBER 1920*

## PHYSIOLOGICAL ISOLATION BY LOW TEMPERATURE
## IN BRYOPHYLLUM[1]

C. M. CHILD AND A. W. BELLAMY

(WITH SIX FIGURES)

### Introduction

It has long been known that certain relations of dominance or control and subordination exist between different parts of plants. Among these relations the most general is the dominance of the chief growing tip over other buds, branches, etc.; that is, over other growing tips. That these relations are not specific for particular parts of the plant is shown by the fact that the growth and development of a bud, for example, may be inhibited by the chief growing tip of the plant, by a branch, or by a leaf. Moreover, the fact has been established that the dominance of one part over another is associated in some way with the metabolic activity of the dominant part. When this is inhibited, for example, by inclosure of a growing tip in an atmosphere without oxygen, or in plaster, the effect on other buds is essentially the same as if the growing tip had been removed; that is, it is no longer dominant, but may regain its dominance when the inhibiting conditions are removed and it again becomes active.

In various publications (CHILD 1–6) it has been pointed out that this relation of dominance and subordination is not peculiar to plants, but that similar relations exist in animals. In these

[1] From the Hull Zoölogical Laboratory, University of Chicago.

and other papers it has also been maintained on the basis of various lines of evidence that this relation originates in a difference in rate of fundamental metabolic reactions, such differences of course being associated with differences in protoplasmic condition. These differences appear in the form of gradations in physiological condition, which have been called metabolic or physiological gradients. Since a discussion of the nature and origin of these gradients and the evidence on which the conclusions are based has recently appeared (CHILD 6), extended discussion of these matters is not necessary at this time. In such a physiological gradient the dominant region is primarily the region of highest metabolic rate, of greatest physiological activity. The evidence indicates further that the relation between dominant and subordinate parts is primarily transmissive, rather than transportative in character; that is, that the dominance of a particular part is primarily dependent upon dynamic changes transmitted from it to other parts, rather than upon the transportation from the one part to the other of substances in mass. Since this dynamic effect of a region of high metabolic rate upon other parts is a factor in determining the metabolic rate of the parts affected, and since in protoplasm without highly specialized conducting paths it decreases with increasing distance from the region of high rate, the result of the establishment of such a region of high rate, however brought about, is the development of a more or less definite physiological gradient. The physiological axis in its simplest terms is such a gradient, determined in relation to a region of high activity which is the dominant region of the axis.

It has been found, largely through the work of HYMAN and BELLAMY, which is not yet published, that gradients in electrical potential and electric currents resulting from them are characteristic features of these physiological gradients. Discussion of the significance of these bioelectric currents is impossible here, but many facts make it highly probable that they are the factors primarily concerned in transmission of excitation (LILLIE 8–13), and that they play a fundamental part in determining and maintaining the physiological gradients which arise in relation to regions of high metabolic rate. If this is true, the dominance of

one part over another is probably primarily a matter of the differences in electric potential and resulting currents. In general, the "high end," the dominant region of a physiological gradient, is externally electro-negative to other levels of the gradient, and in this respect it is similar to a region of excitation, which is also externally electro-negative to less excited or unexcited regions. From this viewpoint the physiological gradient may be regarded, at least tentatively, as the physiological expression or effect of the potential gradient and the resulting currents which arise in relation to a region of high metabolic rate. In fact, the physiological gradient in its simple form shows all the characteristics of an excitation-transmission gradient in protoplasm. In all except the simplest animals a nervous system with definite morphological conducting paths develops as an expression and resultant of the physiological gradients, and after definite nervous relations are established between parts, the dominance of a particular region, for example, the head, is no longer necessarily dependent upon the persistence of the metabolic conditions which originally determine its dominance. While the rate of metabolism concerned in the initiation of a nervous impulse is undoubtedly high, its total amount may be exceedingly small, yet the impulse may determine an enormous amount of metabolism in the organ affected by it.

In plants, however, no nervous system develops, and the relations of dominance and subordination apparently depend throughout life upon essentially quantitative physiological differences of the same sort as those in which the relation originates. The nervous structure of higher animals is capable of conducting impulses for long, perhaps for indefinite distances; but in the less highly specialized protoplasms of the simpler animals and the plants the dynamic effects of excitation undergo a decrement with increasing distance from their point of origin. Such a decrement determines the existence of the physiological gradient, and it is evident that under such conditions physiological dominance of any part must be limited in range, and that therefore the possibility of what the senior author has called physiological isolation (1–6), that is, of escape or isolation from such dominance without physical separation of parts, exists. Theoretically physiological isolation

may occur in four ways: first, growth in size of the organism may
bring some part of it beyond the range of the dominant region;
second, since dominance is primarily dependent upon the metabolic
activity of the dominant region, a decrease in this activity, how-
ever brought about, will decrease the range of dominance and may
bring about physiological isolation in more distant parts without
increase in size; third, if physiological dominance is dependent
upon transmission of electric or other dynamic effects through
protoplasm, physiological isolation must result from blocking the
passage of such effects; fourth, the subordinate part may be
directly excited by external factors to such a degree that the action
of the dominant part upon it is no longer effective, for example,
stated in electrical terms, it may itself give rise to electric currents
in the opposite direction from those in the dominant region and
compensating them.

The physiologically isolated part behaves essentially as it
would if the dominant part had been removed, or it itself separated
from the dominant region. If its growth and development have
previously been inhibited, it begins to grow and develop. If it
represented a differentiated part of the body, as in many animals,
it reacts in the simpler forms by losing this differentiation and
may give rise agamically to a new individual.

Physiological isolation and consequent development of new
parts or individuals as a result of growth is a familiar phenomenon
in both plants and animals. The experimental decrease in the
activity of the growing tip by inclosing it in an atmosphere lacking
oxygen, or in plaster, as well as many cases of the inhibiting action
of external factors in nature on growing tips afford numerous
examples among plants of the second kind of physiological isola-
tion. The fourth type of isolation appears in cases in which a
bud may be made to grow in spite of the inhibiting action of a
growing tip or other part, by subjecting it to external conditions
which increase its activity. Such isolation may be brought about
in some plants, particularly in the buds farthest away from the
dominant region. In *Bryophyllum*, for example, the buds in the
notches of the lower leaves will often develop under favorable
external conditions.

As regards the third type of physiological isolation, by the blocking of passage of the action or effect, whatever its nature, less is known.   McCallum (21) obtained some results along this line in plants by means of local anaesthesia, and it is a familiar fact that the passage of the nerve impulse can be blocked by an anaesthetized or a cooled region, as well as by various other means. For a long time one of us has had in mind experimentation along this line with plants, and Loeb's (14–20) recent work on *Bryophyllum* constituted an additional stimulus.   Finally, in 1918–1919, experimentation was begun in the attempt to determine whether physiological isolation could be brought about by a local reversible action on some part of the connecting path between dominant and subordinate parts.   Since it seemed desirable to avoid the use of anaesthetics or other chemical agents which might enter the plant tissues and be transported in one direction or another, low temperature was used as the blocking factor.

## Method and apparatus

The low temperature was obtained by a current of cold water flowing through a block tin pipe of $\frac{1}{8}$ or $\frac{3}{16}$ inch inside diameter, this pipe being bent into loops or coils of the proper diameter and length to surround the portion of the plant to be cooled.   In this way various lengths of stem, from 1 cm. to a whole internode or more, could be cooled.   In preparation of the plant, the region to be subjected to the low temperature was first wrapped in tin-foil, and the loop or coil of pipe, supported by clamps attached to ring stands, was fitted about it in such manner that it was not in direct contact with the plant at any point, the space between stem and pipe being usually 0.5–1 cm.   This space was then packed lightly with moistened absorbent cotton to keep the temperature as constant as possible, and finally the whole coil was wrapped closely in non-absorbent cotton to protect it from the outside temperature.   In many experiments a thermometer was also inserted in the coil, but it was found that so long as the temperature of the water passing through the coil remained constant there was practically no change inside.   In a part of the experiments the temperature of the water used was maintained in a tank by

means of a refrigerating brine coil under thermostatic control. The freezing point of the water in the tank was slightly lowered by the addition of alcohol in order to avoid accumulation of ice about the brine coil. The circulation of water from and to the tank was maintained by an electrically driven pump. The flow from the tank was led to a horizontal feeder pipe 6 ft. above the experimental table, and in this feeder six outlets were tapped about 30 cm. apart. At the table level a similarly tapped collector pipe returned the water to the tank. The flow for each plant was led from the feeder by one of the six insulated outlets, through rubber tubing to the loop or coil about the plant, through the coil and back to a corresponding tap on the collector pipe. With this apparatus as many plants as there were pairs of outlets (supply and return) could be placed under experimental conditions at the same time. Each supply pipe was fitted with a valve, making it possible independently to regulate or stop entirely the flow of cold water through any of the six coils. By means of these valves temperatures differing by several degrees could be maintained in different lines without affecting appreciably the temperature of the general supply. All exposed metal piping was covered with non-conducting material. During the winter months, while the temperature of the city water was so low that it could be used directly, a second similar system was also arranged for use with the city water. Each system was supplied with six connecting lines, so that twelve experiments could be conducted at the same time. With a little care it was possible to control within about 1° C. the temperature to which the cooled region was subjected. This temperature apparatus was devised by the junior author, and for its maintenance in proper working order during the experiments he is largely responsible.

The temperatures found to be effective for the purpose ranged from 2.5 to 6° C., according to the species of plant used and the region subjected to cooling. In the bean seedling, in which regions of the main stem were cooled, the more basal levels of the stem required a lower temperature than more apical levels to bring about physiological isolation and outgrowth of buds below the cooled zone.

Thus far experiments have been made chiefly with three species of plants: *Bryophyllum calycinum*, in which physiological isolation and outgrowth of buds in the notches of the leaves was brought about by cooling a region of the petiole; seedlings of *Phaseolus multiflorus*, the scarlet runner bean, in which isolation and outgrowth of axillary buds were brought about by cooling a region of the main stem between the buds to be isolated and the chief tip of the plant; *Saxifraga sarmentosa*, in which the isolation and development of the runner tip into a new plant was brought about by cooling a zone of the runner below its tip. A brief report of the results of these experiments has already appeared (CHILD and BELLAMY 7). The present paper is devoted to the experiments on *Bryophyllum*.

## Experiments

The individual plants used ranged from 0.6 to 1.3 m. in height. Leaves from the upper half of the plant were selected for experiment in nearly all cases, particularly in the larger, older plants, in which the lower leaves are often in poor condition, or, when the plants have been kept in moist air, show more or less outgrowth of the buds during the winter in the intact plants. Various preliminary experiments were performed in order to determine to what extent physiological isolation might be brought about by external conditions acting directly on the leaves while still attached to the intact plant. By arranging bowls or jars containing water on ring stands about the plant in such a way that particular leaves were more or less completely submerged, it was found that at least during the winter and early spring months some of the leaf buds would grow in some cases, but to a greater extent on leaves at lower than on those at higher levels of the plant.

Again, a sudden rise in temperature from 15 to 25° C. in saturated atmosphere would usually induce outgrowth of some buds on leaves of the middle and lower levels of the plant, but not on those near the main tip. Such outgrowth usually consisted only of roots, and these were often inhibited after a few days.

Direct injury to the petiole, for example, compression by a screw clamp or by a cut partially through the petiole, was usually

effective, if sufficient in degree, in inducing more or less outgrowth of leaf buds, provided the leaf concerned was in saturated air or water. Slight superficial injury, even though it extended completely around the petiole, had little or no effect. An injury to the petiole of one leaf sufficient to induce outgrowth of some of the buds of that leaf usually induced outgrowth of some buds on the opposite leaf, and sometimes also on leaves of the next node above or below, if these were in moist air or water. Such mechanical injury by partial section of the petiole or by compression, however, was less effective than low temperature, unless the injury was sufficient to interrupt physiological continuity to a very considerable degree. Cuts half way through the petiole, for example, brought about development of some buds on the leaf, but usually of only a few, and in the case of lateral cuts the buds growing were not necessarily on the same side as the cut. Mechanical compression of the petiole by a screw clamp gave similar results. Fig. 1
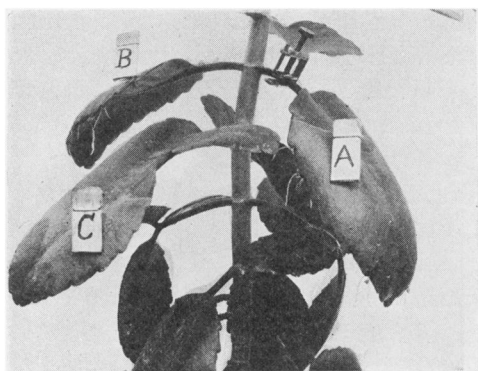


FIG. 1

shows a case in which the petiole of the leaf $A$ was compressed to half its thickness by the screw clamp. The leaf $A$, the opposite leaf $B$, and one leaf $C$ of the next pair below were partially submerged. On leaf $A$ five buds grew out of eighteen submerged; on leaf $B$, seven out of seventeen submerged, but they are less advanced than those of $A$. On leaf $C$ there is slight growth of four out of seventeen buds submerged, but this growth consists merely of short roots and is not visible in the figure. In another similar experiment slightly less compression resulted in outgrowth of only three buds out of twelve submerged on the leaf with compressed petiole, and no growth in $B$ and $C$. Comparison of these results with those obtained by low temperature described later

shows that the low temperature is far more effective, even though no visible injury results, than mechanical compression.

These various experiments show very clearly that the dominance of the chief growing tip of *Bryophyllum* may be overcome to some extent at levels below the most apical five or six nodes without separating the leaf from the plant or inhibiting the chief tip, and in some cases by merely placing the leaf in water or in moist air with its petiole and attachment intact. Such isolation, however, usually results in development of only a part or a few, often of only one or two of the buds on a leaf. Whether physiological isolation of the leaf buds will occur as readily during the summer months has not yet been determined. The fact that injury to the petiole of one leaf may, if sufficient in degree, induce growth of buds in the opposite leaf and often in the leaves of adjoining nodes, shows further that the inhibition of growth of buds in any leaf is due, not merely to the chief tip of the plant, but to the opposite leaf and to some extent to other leaves also. This fact has also been shown by earlier work and more recently by LOEB's experiments.

The results of cooling a zone of the petiole, however, are much more striking. In these experiments a portion of the petiole 2–3 cm. in length is subjected to the low temperature, the rest of the petiole and leaf being exposed to room temperature, and the leaf blade more or less completely submerged in water, as indicated in fig. 2, or by placing an open bowl of water in such position that the leaf rests in the water. The succulent tissues of *Bryophyllum* are very susceptible to injury by continued pressure, and care must be taken that the low temperature coil does not touch the petiole, that the packing of the coil is not too tight, and that the petiole does not touch the edge of the jar or bowl in which the leaf is submerged. In some experiments, particularly the earlier, injury of the petiole resulted from one of these causes; but although the results of the experiments on mechanical injury indicate that the slight injuries thus produced had little or no effect on the leaf buds, only those experiments in which no visible mechanical injury of the petiole was found after removal of the low temperature zone are regarded as entirely satisfactory.

In room temperature of 20–25° C., growth of the leaf buds usually became visible three to four days after the low temperature coil was placed, the first indication being the outgrowth of one or
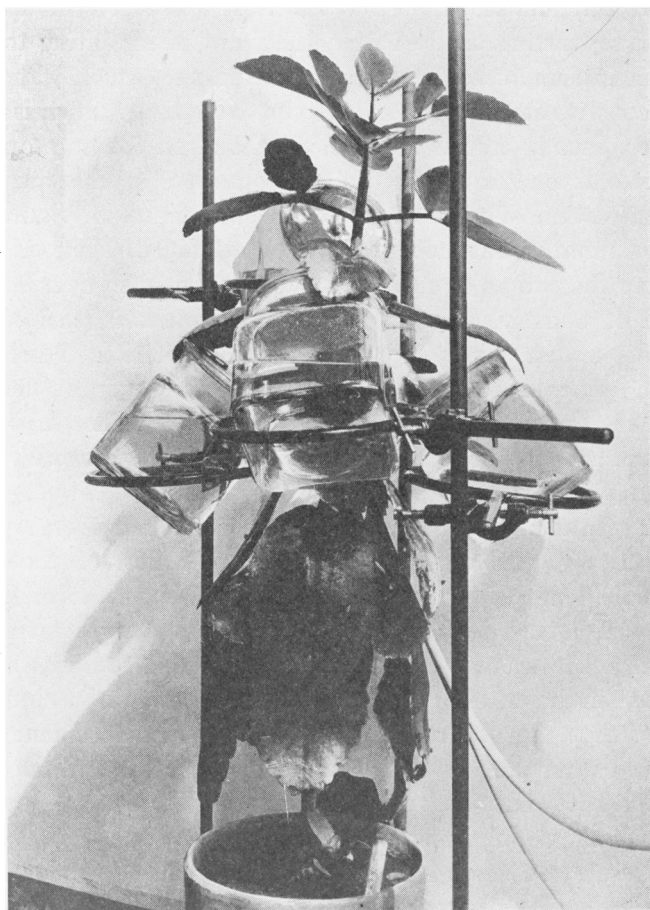


FIG. 2

more roots. If the low temperature coil was removed at this time, shoots usually did not appear, or appeared in only a few notches, the others being inhibited. If the low temperature coil remained in place six to eight days, shoots as well as roots were

usually clearly visible before its removal, and growth always continued afterward in at least a considerable number of notches. When the coil remained on the petiole for ten days or more, death of some of the epidermal cells usually occurred, although internally the petiole might be completely intact and to all appearances in good condition. Probably such superficial injuries were without effect, but cases in which they occurred were not regarded as conclusive.

In all, twenty-eight low temperature experiments were performed with *Bryophyllum* leaves attached to the plant. In fifteen of these there was some injury to the petiole, but in the other thirteen no injury was apparent. In all these experiments not only the experimental leaf, but the opposite leaf, and usually one or more leaves of nodes above and below the node of the experimental leaf, were more or less completely submerged, in order to determine to what extent these leaves were also affected. Usually all, or nearly all, buds on the submerged portion of the experimental leaf and in most cases those of the leaf opposite developed. On some leaves individual buds had been killed or injured by plant lice, from which the plant could not be kept entirely free. As far as possible, leaves were selected for experiment on which all the buds were apparently intact, but occasionally such buds failed to grow.

In order to determine whether cooling of a zone of the petiole stopped the flow of water to the leaf, experiments were performed in which the experimental leaf remained in air of medium humidity, instead of being submerged. Such leaves did not wilt, but remained fresh and in good condition, while leaves separated from the plant and exposed to the same atmosphere showed distinct wilting in the course of a few days. Evidently the cooled zone does not appreciably affect the flow of fluids to the leaf. The following descriptions and figures of typical experiments will serve to show the results attained.

Series 35, March 12, 1919.—Temperature of 2.5–3° C. was placed on petiole of one leaf of the eighth pair below the tip. The terminal leaflet of this leaf, of the opposite leaf, and one leaf of the ninth pair were submerged. Growth was visible in both leaves

of the eighth pair after three days, but none in the leaf of the ninth pair. After five days the cooled zone was gradually brought to room temperature and the coil removed. Fig. 3 shows the condition of the plant twelve days after the beginning of the experiment. The experimental leaf is the member on the left of the figure of the pair showing bud development. In this and the opposite leaf all buds which were submerged show vigorous outgrowth. In the leaf of the ninth pair ten buds out of twelve submerged developed, but only three of these produced distinct shoots,
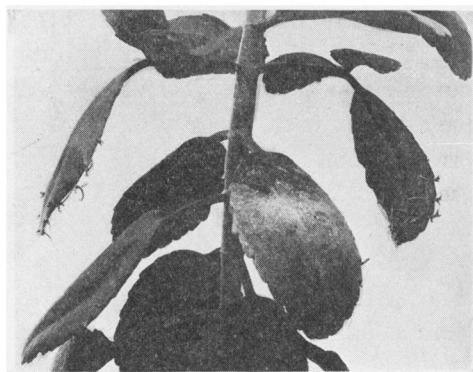


FIG. 3

and all are much retarded as compared with those of the leaves above. Fig. 3 does not show the roots on this leaf, since they are on the under side.

Series 44, March 30, 1919.—Temperature of 3–4° C. on petiole of one leaf ($A$) of seventh pair below tip. Opposite leaf ($B$) and both leaves ($C$ and $D$) of sixth pair in water. The leaf $D$ was in water eight days preceding the temperature experiment, but during that time showed no development of buds. After seven days the cooled zone was gradually brought to room temperature and the coil removed. The experimental leaf ($A$) showed growth in ten notches, $B$ in two notches, $C$ in none, and $D$ in five notches. During the following week three days of high temperature in the greenhouse occurred, and this may have aided the development of some further buds. The condition of the four leaves eighteen days after the beginning of the experiment is indicated in fig. 4. In $A$ every bud submerged has developed; in $B$ nine buds out of fourteen submerged have developed, but only three of them have gone beyond the earliest stages; in $C$ eight buds out of sixteen submerged show some development, but all except two have been inhibited in early stages; in $D$ four buds out of fifteen submerged have developed

and show shoots as well as roots. Fig. 4 shows clearly the difference between $A$ and the other leaves, although it does not show all the development in $B$, $C$, and $D$. The more advanced development of the four buds in $D$ suggests that the eight days in water preceding the temperature experiment may have had some slight effect in the way of isolation, although it did not lead to visible development.

Series 45, April 4, 1919.—Temperature 2.5–3° C. on one leaf $(A)$ of sixth pair below tip; $B$, opposite leaf, $C$, one leaf of seventh pair, $D$, one leaf of eighth pair, and $E$, one leaf of fifth pair also in water. After seven days the cooled zone was gradually brought to room temperature and the coil removed. Fig. 5 shows the plant seventeen days after beginning of the experiment. In $A$ fourteen buds out of fifteen submerged developed and formed vigorous shoots; in $B$ seven out of fifteen submerged
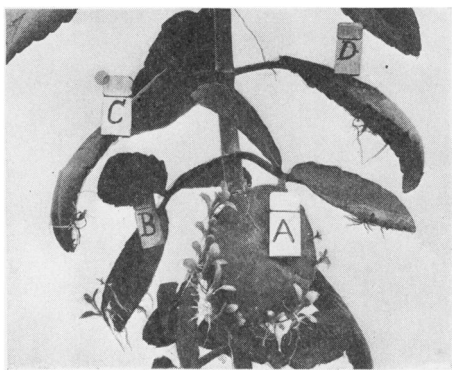


FIG. 4

developed to some extent, but produced only roots; in $C$ eleven out of fifteen submerged showed some development, but only two produced shoots; $D$ showed no growth; in $E$ all buds submerged (thirteen) developed, but only six produced shoots. The growth on leaf $C$ is not well shown in the figure. The slight development in the leaf $B$ of this experiment is unusual. In most cases the mate of the experimental leaf shows almost or quite as much growth as the experimental leaf itself.

Series 43, March 27, 1909.—Temperature of 2.5–3 C. on petiole of one leaf $(A)$ of eighth pair; only the terminal leaflet in water; $B$, leaf opposite $A$, $C$, one leaf of eleventh pair, $D$, $E$, terminal leaflets of leaves of ninth pair, $F$, one leaf of seventh pair, $G$, one leaf of fifth pair, all in water. After six days the cooled zone was gradually brought to room temperature and the coil removed.

At this time $A$ showed the greatest development, both as to stage and number of buds (eight) developing; $B$ showed two buds developing; $C$ and $D$ none; $E$ six; $F$ one; and $G$ none. Fig. 6 shows the plant fifteen days after beginning of the experiment. At this time in $A$ seventeen buds out of nineteen had developed, and sixteen had produced shoots as well as roots; in $B$ fourteen buds out of sixteen submerged had developed, thirteen with both shoots and roots; in $C$ four buds out of nineteen submerged had developed, all with both shoots and roots; in $D$ two buds out of ten

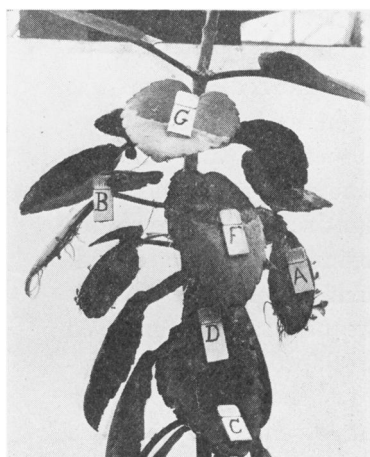

FIG. 5                          FIG. 6

submerged had developed, one with shoot and roots; in $E$ ten buds out of sixteen submerged had developed, all with shoots and roots; in $F$ eight buds out of sixteen submerged had developed, three shoots and roots, five roots only; in $G$ seven buds out of twelve submerged showed some slight development, but only two showed shoots, the others roots only.

In this and other experiments described, some of the isolations on leaves distant from the experimental leaf may not be the result of the cooling, but merely such as occur on most plants at this season, when leaves are submerged. In all the series, however, it is evident that the greatest degree of isolation, both as regards

number of buds developing and degree of development, has occurred in the experimental leaf and the leaf opposite.

In these experiments generally the buds which do not develop distinct shoots as well as roots within a week, either do not develop shoots at all, or give rise to shoots which grow very slowly and often soon cease to grow. Such buds are evidently inhibited to a greater or less degree. Apparently they represent somewhat less active or weaker buds, which react less rapidly than others to the isolating conditions, and so do not advance far enough before the return of the experimental leaf to normal conditions to maintain their growth afterward.

It appears true also that in general buds give rise only to roots when the degree of physiological isolation is relatively slight, while with more complete isolation shoots as well as roots appear. The length of the isolation period is probably also a factor, since the outgrowth of roots begins somewhat earlier than that of the shoots, or at least occurs more rapidly during the early stages. A brief period of isolation gives time for roots to appear, but a longer period is apparently necessary for the shoot to become well started.

## Discussion

It is evident from these experiments that a mere cooling of a zone of the petiole of the *Bryophyllum* leaf without visible physical injury serves to block the inhibiting action of the chief growing tip and other parts upon the buds of that leaf, and also the inhibiting action of the leaf upon the buds of the opposite leaf and other leaves in the same region of the plant. That this cooling does not block the flow of fluids and substances in solution through the vascular bundles is indicated by the experimental fact noted that leaves show no wilting in an atmosphere in which leaves severed from the plant wilt. In the case of the bean seedling, to be discussed in a later paper, this is still more clearly evident, for there the zone of low temperature is placed about the main stem, and all substances passing from below to those parts of the plant above the cooled zone must of course pass through it. In such cases there is no wilting of the parts above the zone, and growth is either not at all inhibited, or, when the region cooled is young

and not fully developed, growth of the parts above may be retarded slightly for two or three days, but soon proceeds normally.   These facts do not support the view which LOEB has advanced that the inhibiting action of the growing tip and of other parts on buds is due to the transportation of inhibiting substances through the regular channels of transportation in the plant.   In these experiments such transportation is not appreciably or only very slightly affected, yet the zone of low temperature is much more effective as a means of physiological isolation than mechanical compression or partial section of the petiole, except when these involve the greater part of the petiole tissues.   Unless we assume that the hypothetical inhibiting substance in some way is rendered inactive by the short cooled zone, we must conclude that the dominance of the growing tip and of other regions over a particular leaf is not dependent upon the flow of substances through the vascular bundles to the leaf, but rather upon some sort of action which is dependent upon the physiological activity of the cells.   When this activity is inhibited by the low temperature, the action is blocked, unless and until some degree of acclimation of the cooled zone occurs.   Such acclimation occurs very readily in the bean seedling, and in many cases a temperature which at first serves as a block becomes ineffective after a few days.   In short, the experiments indicate that the physiological dominance of one region over another in these plants is dependent on some sort of effect transmitted physiologically through the living active protoplasm, rather than upon substances transported by the flow of fluids.

LOEB appears not to distinguish clearly two different aspects of the relations of parts:  the one which is concerned with the conditions that prevent or permit the initiation of development and growth in a subordinate part;  the other which is concerned with the amount of growth or development of the part which may occur after its initiation.   Nutritive factors may play a large part in determining the amount of growth of buds, but there are no reasons for and many against maintaining, as LOEB did in earlier papers, that they initiate it.   Again, the mass of shoots and roots developing from an isolated *Bryophyllum* leaf may show a certain proportion to the size of the leaf, since the amount of certain

nutritive substances available must depend upon the size of the leaf, but such relation tells us nothing concerning the factors which initiate the development.

On the other hand, the assumption of the transportation of inhibiting substances, made in LOEB's later papers, also involves certain difficulties. In the first place, each part which produces such substance or substances must be immune to the action of the substance which it produces, since it is not inhibited by it, yet in the case of growing tips the substance produced by one growing tip inhibits other tips. This presupposes a remarkable specificity of action on the one hand and absence of specificity on the other, and it is difficult to conceive how the hypothetical substance could possess the properties required. Certain assumptions concerning the direction of flow of the inhibiting substances also have no basis in fact and do not agree well with the facts at hand. Certain other objections to the assumption of inhibiting substances scarcely require discussion in view of the work of various botanists and the experiments just described.

In an early paper LOEB (**14,** pp. 251–253) endeavored to show that isolation is not the initiating factor in the outgrowth of buds on the leaf of *Bryophyllum*, and described three experiments to prove his point (see his *figs. 1, 2, 3*). In the first a leaf partially submerged is completely separated from the stem; in the second it remains attached to a piece of stem cut off above and below the node and the opposite leaf is removed, but its axillary bud remains; and in the third the opposite leaf also remains. In the first experiment and in the third the submerged buds develop, in the second they do not, but the axillary bud of the opposite side develops in the absence of its leaf. LOEB maintains that the leaf in the second experiment is more isolated than in the third, but its buds do not grow out, therefore isolation cannot be the factor determining the development of the buds. This conclusion is incorrect and based upon a misconception of isolation. Actually the leaf of the second experiment is less isolated than in the first and third, because in this experiment the axillary bud of the opposite side develops and inhibits the leaf buds. If this growing tip is removed, the buds of the leaf will develop. In the third experiment the

axillary bud is inhibited by its own leaf and there are no active growing tips to inhibit the direct action of the water in inducing bud development in the experimental leaf. Isolation from an active growing tip is the chief factor in the development of the leaf buds, and such isolation exists to a greater degree in LOEB's first and third experiments than in the second, in which the axillary bud of the opposite side starts before the leaf buds of the experimental side. These three experiments, therefore, instead of disproving, as LOEB asserts, that isolation is the factor initiating development of the leaf buds, constitute evidence in support of the conclusion that it is such a factor. Moreover, according to LOEB's later assumption of inhibiting substances, it would seem that isolation must be the initiating factor.

The chief results of the paper are summarized as follows. The cooling of a zone of the petiole of the *Bryophyllum* leaf to a temperature of 2.5 to 4° C. for a few days is a very effective means of inducing the outgrowth of the leaf buds. Usually the opposite leaf and often leaves of adjoining nodes also show more or less development. The passage of fluids to the leaf is not appreciably interfered with by the cooled zone; therefore it seems improbable that physiological isolation of the leaf can be due to the blocking of passage of inhibiting substances transported in these fluids.

UNIVERSITY OF CHICAGO

## LITERATURE CITED

1. CHILD, C. M., Studies on the dynamics of morphogenesis, etc. Jour. Exp. Zool. **11**:187–220; 221–280. 1911.
2. ————, Die physiologische isolation von teilen des organismus. Vort. und aufs. u. Entwickelungsmech. **11**:157. 1911.
3. ————, Individuality in organisms. Chicago. 1915.
4. ————, The gradient in susceptibility to cyanides in the meridional conducting path of the ctenophore, *Mnemiopsis*. Amer. Jour. Physiol. **41**. 1917.
5. ————, Differential susceptibility and differential inhibition in the development of polychete annelids. Jour. Morph. **30**:1–63. 1917.
6. ————, The nature and origin of the physiological gradients. Biol. Bull. **39**. 1920.
7. CHILD, C. M., and BELLAMY, A. W., Physiological isolation by low temperature in *Bryophyllum* and other plants. Science **50**:362–365. 1919.

8. LILLIE, R. S., The conditions determining the rate of conduction in irritable tissues and especially in nerve.   Amer. Jour. Physiol. **34**:414–445. 1914.

9. ———, Conditions of conduction of excitation in irritable cells and tissues and especially in nerve. II. Amer. Jour. Physiol. **37**:348–370. 1915.

10. ———, The conditions of physiological conduction in irritable tissues. III. Amer. Jour. Physiol. **41**. 1916.

11. ———, The formation of structures resembling organic growths by means of electrolytic local action in metals and the physiological significance of that type of action.   Biol. Bull. **33**:135–186. 1917.

12. ———, Transmission of activation in passive metals as a model of the protoplasmic or nervous type of transmission.   Science **48**:51–60. 1918.

13. ———, Nervous and other forms of protoplasmic transmission.   Sci. Mo. **8**:456–474; 552–567. 1919.

14. LOEB, J., Rules and mechanism of inhibition and correlation in the regeneration of *Bryophyllum calycinum*.   BOT. GAZ. **60**:249–276. 1915.

15. ———, Further experiments on correlation of growth in *Bryophyllum calycinum*.   BOT. GAZ. **62**:293–302. 1916.

16. ———, Influence of the leaf upon root formation and geotropic curvature in the stem of *Bryophyllum calycinum* and the possibility of a hormone theory of these processes.   BOT. GAZ. **63**:25–50. 1917.

17. ———, The law controlling the quantity of regeneration in the stem of *Bryophyllum calycinum*.   Jour. Gen. Physiol. **1**:81–96. 1918.

18. ———, The physiological basis of morphological polarity.   Jour. Gen. Physiol. **1**:337–362. 1919.

19. ———, The physiological basis of morphological polarity. II. Jour. Gen. Physiol. **1**:687–715. 1919.

20. ———, Quantitative laws in regeneration. I.   Jour. Gen. Physiol. **2**:297–307. 1920.

21. McCALLUM, W. B., Regeneration in plants.   BOT. GAZ. **40**:97–120; 241–263. 1905.